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High Frequency of Virus-Specific CD8$^+$ T Cells in the Central Nervous System of Macaques Chronically Infected with Simian Immunodeficiency Virus SIVmac251

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Infection with human immunodeficiency virus or simian immunodeficiency virus (SIV) induces virus-specific CD8$^+$ T cells that traffic to lymphoid and nonlymphoid tissues. In this study, we used Gag-specific tetramer staining to investigate the frequency of CD8$^+$ T cells in peripheral blood and the central nervous system of Mamu-A*01-positive SIV-infected rhesus macaques. Most of these infected macaques were vaccinated prior to SIVmac251 exposure. The frequency of $Gag_{181-189}$ CM9 tetramer-positive cells was consistently higher in the cerebrospinal fluid and the brain than in the blood of all animals studied and did not correlate with either plasma viremia or CD4$^+$-T-cell level. Little or no infection in the brain was documented for most animals by nucleic acid sequence-based amplification or in situ hybridization. These data suggest that this Gag-specific response may contribute to the containment of viral replication in this locale.

The central nervous system (CNS) is a target for many microbial and viral infections, including simian immunodeficiency virus (SIV) and human immunodeficiency virus (HIV) (2, 9, 25, 37, 47). CNS infection occurs during primary SIV infection (7), and the degree of CNS pathology varies among animals (6, 17, 26, 39, 48). For chronically infected macaques, a significant decrease in viral RNA is observed in the CNS, even though viral DNA persists (8).

Although many studies have investigated the presence of HIV or SIV in the CNS, less knowledge about virus-specific immune responses in this locale exists (38). CD8$^+$ T cells, including virus-specific cytotoxic T lymphocytes (CTLs), are important for the containment of systemic viral replication (21, 32, 40) and may also play a role in containing viral replication in the CNS. In HIV type 1 (HIV-1) infection, a large number of virus-specific CTLs in the cerebral spinal fluid (CSF) of patients with AIDS dementia complex has been reported (20). In some of these individuals, these CTLs were present at higher frequencies in the CSF than in peripheral blood, leading to the hypothesis that a high frequency of antigen-specific cells in the brain contributes to the development of neurological disorders. Similar mechanisms have been hypothesized for human T-cell leukemia and lymphoma virus type 1 infection (1, 19, 34).

Since SIVmac251 infection of macaques is a widely used model to assess preventive (R. C. Desrosiers, Letter, Science **275:**13, 1997) as well as therapeutic (10) intervention for human AIDS, it is important to assess the frequencies of SIV-specific CTLs in the CNS of SIVmac251-infected macaques. Eleven Mamu-A*01-positive and two Mamu-A*01-negative macaques, chronically infected with the same strain of SIVmac251 (Table 1) for 55 to 237 weeks and with CD4$^+$-T-cell counts of 59 to 1,853, were euthanized. Viral replication in nine of 11 Mamu-A*01-positive animals was below the detection limit of 2,000 copies per 100 μl of input plasma (<20,000 copies/ml), and normal levels of CD4$^+$ T cells were maintained by five of those nine animals following exposure to SIVmac251 (4, 13, 14, 36, 43). All of these animals were vaccinated with poxvirus-based SIV vaccines either before or after SIVmac251 infection, except for macaque 3077, who received a nonrecombinant vaccine (43).

TABLE 1. Histories, CD4$^+$ T cell levels, and plasma viral RNA levels of macaques at time of euthanasia[a]

Macaque[b]	SIVmac251 inoculation route[c]	Duration of infection (wk)	CD4$^+$ T cells/mm^3	Viral RNA copies/ml of plasma
460	i.r.	127	644	56,290
685	i.v.	108	131	<20,000
22M	i.r.	68	1,140	<20,000
674	i.r.	71	1,853	<20,000
273	i.v.	237	59	<20,000
3076	i.v.	137	278	<20,000
3075	i.v.	145	332	<20,000
3057	i.v.	55	1,115	<20,000
3077	i.v.	135	371	190,460
679	i.r.	71	1,205	<20,000
26M	i.r.	71	1,081	<20,000
23M	i.r.	71	390	2,310,080
480	i.v.	235	159	2,086,280

[a] The macaques were previously immunized with ALVAC-SIV (macaque 460) (36), NYVAC-SIV (macaques 480, 674, 685, 273, 3076, 3075, and 3057) (4, 13, 43), or DNA plus NYVAC-SIV (22M, 23M, 26M, and 679) (4, 13, 43).
[b] Boldface numbers indicate Mamu-A *01-positive macaques.
[c] i.r., intrarectal. i.v., intravenous.

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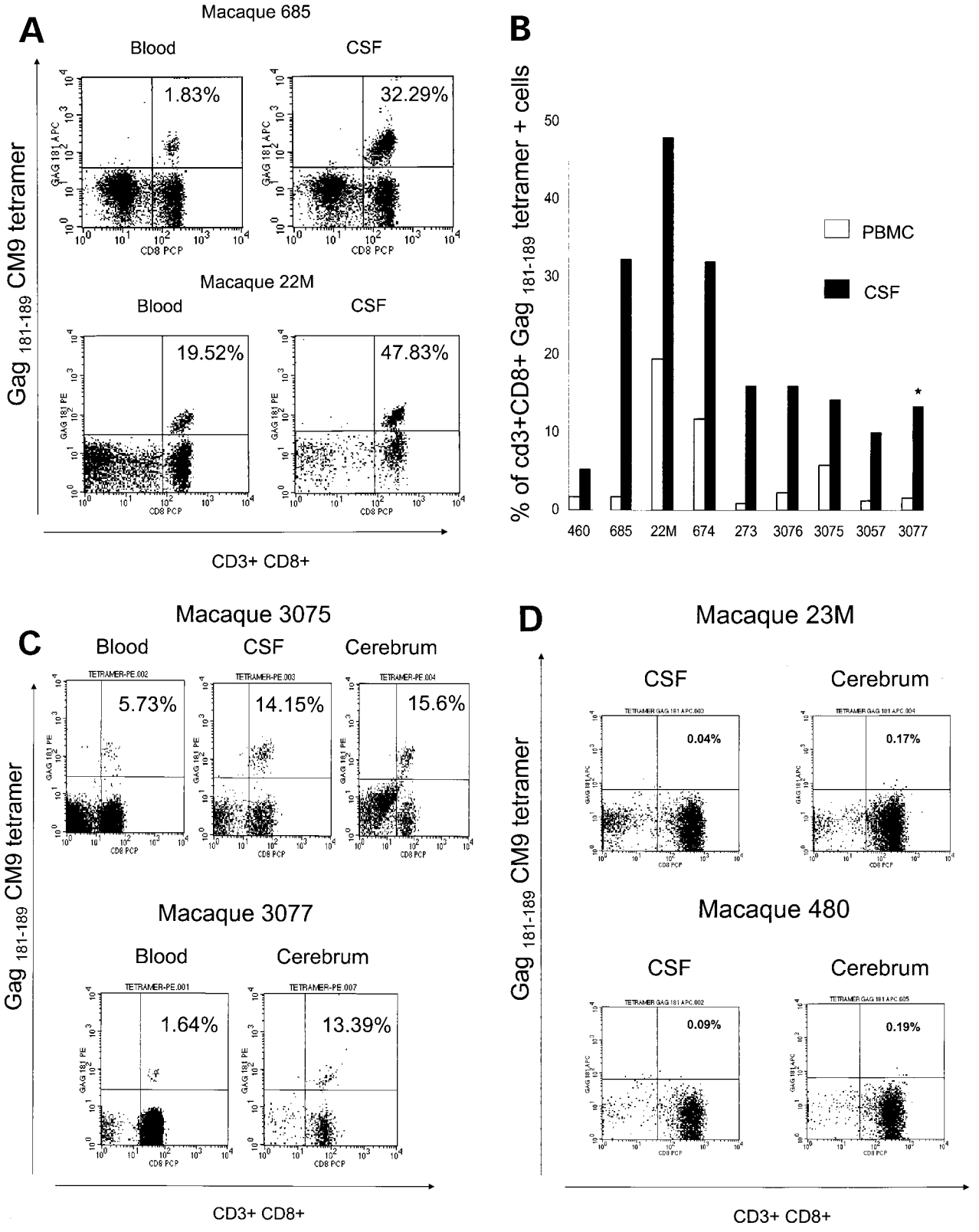

FIG. 1. Frequency of tetramer-positive $CD8^+$ T cells in blood, brain, and CSF of Mamu-A*01-positive and -negative macaques chronically infected with SIVmac251. The typical yield of T cells in CSF and brain ranged from 2,522 to 20,425 and 3,257 to 10,017, respectively. Lymphocytes from blood, CSF, and brain were stained with anti-CD3, anti-CD8, and $Gag_{181-189}$ CM9 tetramers. (A) Representative examples of a dot blot demonstrating the $Gag_{181-189}$ CM9-positive $CD3^+$ $CD8^+$-T-cell population in the blood and CSF of macaques 685 and 22M. (B) Graphic representation of the percentages of tetramer-positive cells within the $CD3^+$ $CD8^+$ population of 9 of the 11 Mamu-A*01-positive macaques summarized in the histograms. ★, CSF was not available, and the data represent the frequency of this population in the brain. (C) Lymphocytes isolated from the brains of macaques 3075 and 3076 were stained with anti-CD3, anti-CD8, and $Gag_{181-189}$ CM9 tetramers. The numbers represent the percentages of tetramer-positive cells within the $CD3^+$ $CD8^+$ population. (D) $Gag_{181-189}$ CM9 tetramer staining of the CSF and cerebra of the Mamu-A*01-negative macaques 23M and 480.

CSF cells were collected by centrifugation at 200 × *g* for 7 min and, after removal of supernatant, were resuspended in Hanks balanced salt solution. Brain lymphocytes were collected as previously described (27). Ex vivo peripheral blood mononuclear cells (PBMCs), CSF, and brain lymphocytes were stained with anti-CD3 (fluorescein isothiocyanate; BD Pharmingen, San Diego, Calif.), anti-CD8 (PerCP; BD Immunocytometry Systems, San Jose, Calif.) monoclonal antibodies (monoclonal Abs), and $Gag_{181\text{-}189}$ CM9 tetramer (phycoerythrin or allophycocyanin (APC) conjugated). In the case of PBMCs, approximately 10^4 $CD3^+$ $CD8^+$ T cells were analyzed. The number of $CD3^+$ $CD8^+$ cells obtained from CSF ranged from 1,244 to 15,383. In the case of macaques 273, 3075, 23M, 480, and 3077, $CD3^+$ $CD8^+$ T lymphocytes were also isolated from the brain.

PBMCs and cells from CSF and the brain were stained with the $Gag_{181\text{-}189}$ CM9 tetramer, and the number of tetramer-positive T cells within the $CD3^+$ $CD8^+$ population was quantitated by flow cytometry. The frequencies of $Gag_{181\text{-}189}$ CM9 tetramer-positive cells were much higher in CSF than in the blood of the SIVmac251-infected macaques, as demonstrated for two of them in Fig. 1A; this enrichment was observed in the CSF of all macaques studied, regardless of the plasma viremia or the level of $CD4^+$ T cells (Fig. 1B). The average frequency of tetramer-positive $CD3^+$ $CD8^+$ lymphocytes in CSF was 21.68% (range, 5.22 to 47.83%) in comparison to 5.61% (range, 0.88 to 19.52%) in peripheral blood. In three of these macaques, 685, 22M, and 674, $Gag_{181\text{-}189}$ CM9-specific $CD8^+$ T cells represented one-third of the entire $CD3^+$ $CD8^+$-T-lymphocyte population, and in these three animals, the relative percentage of $CD4^+$ T cells in CSF was much less than that in peripheral blood, suggesting an enrichment of total $CD8^+$ T cells in CSF (Fig. 1B and data not shown).

In macaques 3075, 3077, and 273, small numbers of lymphocytes were also isolated from brain tissue. The frequency of tetramer-positive $CD8^+$ T cells was assessed in the $CD3^+$ $CD8^+$ population of two of the macaques, 3075 and 3077. The frequency of Gag-specific $CD8^+$ T cells was again higher in the brain tissue than in the blood of both macaques (Fig. 1C). In the case of animal 3075, the levels of T cells in both CSF and the brain were sufficient enough to compare the relative frequencies of tetramer-positive T cells. Comparable frequencies of $Gag_{181\text{-}189}$ CM9-positive T cells in the brain and CSF (14 to 15%) were demonstrated, and, as expected, the frequencies were approximately threefold higher than that in blood (Fig. 1C). The tetramer-staining $CD3^+$ $CD8^+$-T-cell population was specific, as demonstrated by the lack of detection of tetramer-positive cells in the CSF and brains of the two Mamu-A*01-negative macaques chronically infected with SIVmac251, macaques 23M and 480, included in the study (Fig. 1D).

To assess the functionality of Gag-specific $CD8^+$ T cells, their ability to produce tumor necrosis factor alpha (TNF-α) and gamma interferon (IFN-γ) following antigen-specific stimulation was measured by intracellular cytokine staining of lymphocytes obtained from the CSF of macaques 26M and 679 and the brains of macaques 273 and 3077. Lymphocytes isolated from the CSF or brain were stimulated with the $Gag_{181\text{-}189}$ CM9 peptide for 6 h (5 h in the presence of brefeldin A) at 37°C and stained with either anti-CD3 fluorescein isothiocyanate (BD Pharmingen), anti-CD8 PerCP (BD Pharmingen), or anti-CD8β phycoerythrin (Beckman Coulter, Miami, Fla.) and either a mixture of APC-conjugated anti-TNF-α and anti-IFN-γ Abs (BD Pharmingen) or each Ab alone. In the CSF of the Mamu-A*01-positive macaques 26M and 679, a sizeable $CD3^+$ $CD8^+$-T-cell population produced TNF-α as well as IFN-γ following stimulation with the $Gag_{181\text{-}189}$ CM9 peptide (Fig. 2A and B). Similarly, a sizeable portion of $CD8^+$-T-cell-producing cytokines was found in the brain of macaque 273 following $Gag_{181\text{-}189}$ CM9 peptide stimulation (Fig. 2C). In the case of macaque 3077, the number of events observed was too low for analysis (data not shown).

The high frequencies of antigen-specific cells in CSF and the brain suggested ongoing viral replication in the CNS and brain. To address this, we isolated cellular RNA from brain tissue from macaques 460, 674, 3075, 3076, 3077, and 273 and performed SIV RNA amplification by nucleic acid sequence-based amplification. Our assay detected no viral RNA in the brain tissue (assay sensitivity, 500 copies of RNA/input) (data not shown).

To confirm this finding by an independent assay, brain tissue sections from macaques 3075, 3076, 3077, and 273 were analyzed for the expression of viral RNA by in situ hybridization. Formalin-fixed, paraffin-embedded tissues were stained for SIV viral RNA by a previously described method (15). Briefly, the sections were deparaffinized and rehydrated in water, pretreated with 0.2 N HCl and proteinase K, and hybridized overnight at 50°C with either a sense or an antisense SIVmac239 digoxigenin-UTP-labeled riboprobe. All probes were used at a final concentration of 1.75 ng/μl, and the hybridized sections were washed in standard posthybridization buffers and RNase solutions (RNase A, R6513 [Sigma-Aldrich, St. Louis, Mo.]; RNase T1, 109-193 [Roche Molecular Biochemicals, Indianapolis, Ind.]). The hybridized sections were blocked with 3% normal sheep and 3% horse serum in 0.1 M Tris (pH 7.4) and then incubated with the primary Ab, sheep anti-digoxigenin-alkaline phosphatase (1:500; Roche Molecular Biochemicals), in serum-blocking buffer for 1 h. The sections were rinsed with Tris buffer and reacted with NBT-BCIP (Nitro Blue Tetrazolium–5-bromo-4-chloro-3-indolylphosphate; Vector Laboratories, Ltd., Peterborough, England) for 10 h. The samples were rinsed with distilled water, counterstained with 0.1% nuclear fast red (Sigma-Aldrich), washed, dehydrated, and coverslipped with Permount (Fisher Scientific, Pittsburgh, Pa.). Stained sections were viewed with a Zeiss Axiophot microscope and photographed with a CoolSNAP digital camera.

Rare virus-expressing cells were observed in brain tissue sections from macaque 273 but not in sections from the other macaques studied (Fig. 3 and data not shown), providing support to the results obtained by the use of nucleic acid sequence-based amplification to quantitate SIV RNA in the brain. Collectively, these data suggest that the high frequencies of functional antigen-specific $CD8^+$ T cells may contribute to CNS viral replication containment.

In this report we demonstrate that in the CNS of chronically SIVmac251-infected animals, the frequency of $CD8^+$ T cells directed against the dominant SIVmac251 $Gag_{181\text{-}189}$ CM9 epitope is consistently higher than the frequency of $CD8^+$ T cells in blood. The levels of tetramer-binding cells with this Gag specificity in peripheral blood, spleen, lymph nodes, bone marrow, liver, and gastrointestinal and urogenital tracts of

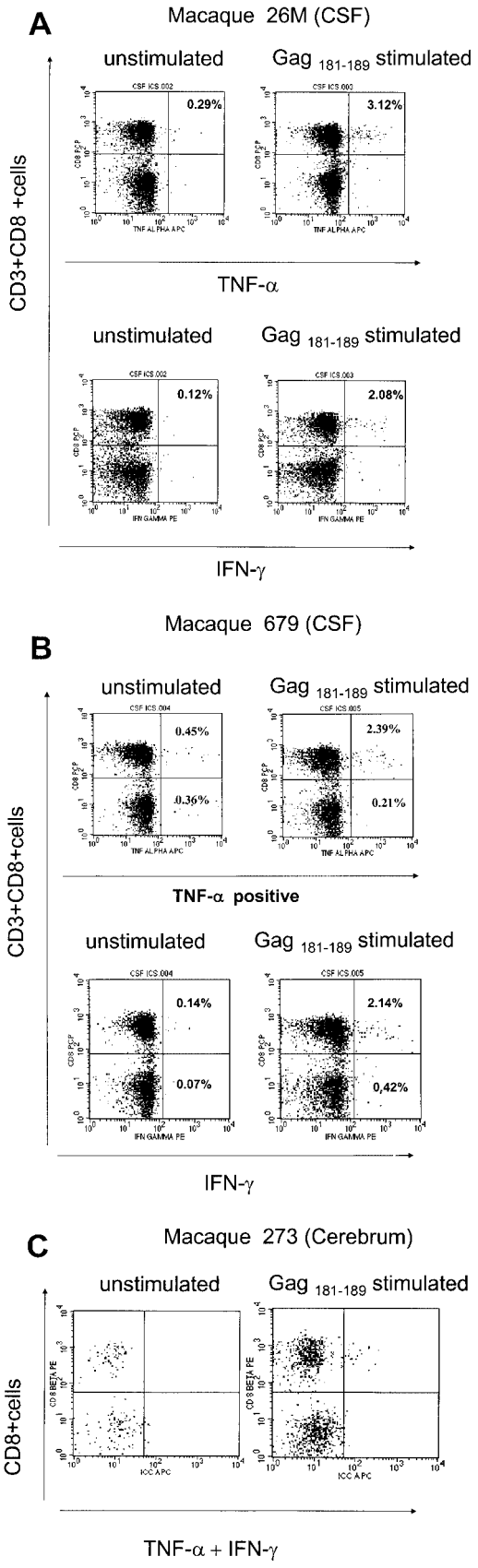

FIG. 2. Functionality of virus-specific $CD8^+$ T cells in CSF and brain. (A and B) For the CSF of animals 26M and 679, 6,224 and 7,726 $CD3^+$ cells were analyzed, respectively. (C) For animal 273, fewer cells from the cerebrum were analyzed (1,500 $CD8\beta^+$ T cells).

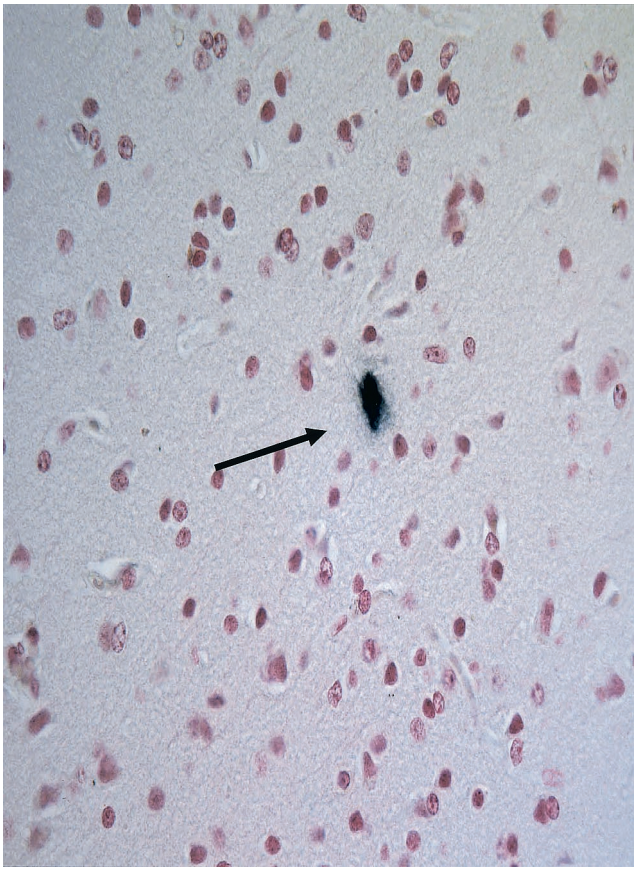

FIG. 3. Detection of viral RNA by in situ hybridization in the brain of macaque 273. An arrow indicates a rare virus-expressing cell.

acutely and chronically SIVmac251-infected macaques have already been extensively analyzed (12, 24, 41, 42, 45). For Mamu-A*01-positive macaques, $Gag_{181-189}$ CM9 tetramer-positive $CD8^+$ T cells emerge in blood at week 2 to 3 after exposure to SIVmac251 and are found as early as 4 weeks postinoculation in secondary and tertiary lymphoid organs (13, 24). The frequency of these CTLs during chronic SIV infection in different compartments varies among animals (12). However, for the majority of chronically infected animals, the frequencies of this response are comparable in peripheral blood and lymph nodes and have often been found to be higher in the gastrointestinal and urogenital tissues, spleen, bone marrow, and liver (12, 41). Here we demonstrate that in all the Mamu-A*01-positive macaques studied, larger numbers of $Gag_{181-189}$ CM9-specific CTLs could be found in the CNS than in peripheral blood (Fig. 1B).

For HIV-1 or SIV infection, the finding in CSF of large numbers of HIV-1-specific CTLs that recognize epitopes different from those recognized by blood CTLs (20, 46) suggests specific local induction or recruitment of antigen-specific cells in CSF. However, other studies demonstrate that T cells can infiltrate the CNS independently of their antigenic specificity

(16). Moreover, even naïve T cells are capable of crossing the brain-blood barrier, but in order to survive in the brain environment, they need to be activated by encountered antigens (23). Indeed, as some have reported, brain $CD8^+$ T cells are comprised mostly of cells presenting an activated and effector phenotype (3, 27, 44). Our data for SIVmac251-infected macaques support the notion that virus-specific $CD8^+$ T cells may be either induced in, or recruited to, the brain. Whether these cells play a role in restricting viral replication in this locale remains to be ascertained.

Antigen-specific $CD8^+$ T cells in the CNS have been suggested as the culprit for tissue damage in cases of SIVmac251 infection as well as in cases of human T-cell leukemia or lymphoma virus type 1-associated myelopathy/tropical spastic paraparesis (1, 19, 35) and mouse hepatitis virus strain JHM infection (28). The presence of memory $CD8^+$ T cells in the brain has been correlated with functional and histopathological abnormalities (27). Despite a high frequency of virus-specific $CD8^+$ T cells in the CNS, none of the macaques studied here had overt symptoms of neurological dysfunction at the time of euthanasia (data not shown). In four out of five macaques with high frequencies of tetramer-binding $CD8^+$ T cells, the cerebral tissues were essentially normal, and in one macaque (3077), a slight increase in perivascular lymphocyte infiltration was found (data not shown). It is possible that these cells are not fully functional in vivo, thereby explaining the lack of tissue damage.

A high frequency of virus-specific $CD8^+$ T cells in the CNS has also been reported for mouse models of acute and chronic infection with influenza virus, JHM, and dengue virus (5, 11, 31, 44). Interestingly, as with SIVmac251 infection, CTLs did not decline in the CNS (unlike systemic CTLs) during the chronic phase of infection even after local viral clearance, suggesting that CTLs remain because of ongoing viral replication, although at times below detectable limits (11, 29). Alternatively, specific CTLs may be selectively recruited to the CNS (22). Other studies have demonstrated that at first, infiltration of the CNS with T cells may be nonspecific (33) but that local expansion of selective T-cell clones may occur as a result of antigen-driven proliferation (18, 30, 33).

Regardless of the underlying mechanism, the data reported here demonstrate that in animals that contain systemic viral replication, restriction of viral replication can also be found in the CNS. Most of the macaques studied here were vaccinated before or after SIVmac251 infection, and several of them were able to contain the viremia for a long time before experiencing a progressive decrease in the number of $CD4^+$ T cells. It is unclear, however, whether vaccination contributed not only to the reduction of plasma viremia but also to the containment of viral replication in the CNS. Studies designed to directly address this issue are warranted, since currently available vaccines do not appear to inhibit viral infection but contribute only to the containment of viral replication.

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REFERENCES

1. **Bangham, C. R.** 2000. The immune response to HTLV-I. Curr. Opin. Immunol. **12:**397–402.
2. **Banks, W. A., E. O. Freed, K. M. Wolf, S. M. Robinson, M. Franko, and V. B. Kumar.** 2001. Transport of human immunodeficiency virus type 1 pseudoviruses across the blood-brain barrier: role of envelope proteins and adsorptive endocytosis. J. Virol. **75:**4681–4691.
3. **Battistini, L., L. Piccio, B. Rossi, S. Bach, S. Galgani, C. Gasperini, L. Ottoboni, D. Ciabini, M. D. Caramia, G. Bernardi, C. Laudanna, E. Scarpini, R. P. McEver, E. C. Butcher, G. Borsellino, and G. Constantin.** 2003. CD8+ T cells from acute multiple sclerosis patients display selective increase of adhesiveness in brain venules: a critical role for P-selectin glycoprotein ligand-1. Blood **101:**4775–4782.
4. **Benson, J., C. Chougnet, M. Robert-Guroff, D. Montefiori, P. Markham, G. Shearer, R. C. Gallo, M. Cranage, E. Paoletti, K. Limbach, D. Venzon, J. Tartaglia, and G. Franchini.** 1998. Recombinant vaccine-induced protection against the highly pathogenic simian immunodeficiency virus SIV_{mac251}: dependence on route of challenge exposure. J. Virol. **72:**4170–4182.
5. **Bergmann, C. C., J. D. Altman, D. Hinton, and S. A. Stohlman.** 1999. Inverted immunodominance and impaired cytolytic function of CD8+ T cells during viral persistence in the central nervous system. J. Immunol. **163:**3379–3387.
6. **Boche, D., E. Khatissian, F. Gray, P. Falanga, L. Montagnier, and B. Hurtrel.** 1999. Viral load and neuropathology in the SIV model. J. Neurovirol. **5:**232–240.
7. **Chakrabarti, L., M. Hurtrel, M. A. Maire, R. Vazeux, D. Dormont, L. Montagnier, and B. Hurtrel.** 1991. Early viral replication in the brain of SIV-infected rhesus monkeys. Am. J. Pathol. **139:**1273–1280.
8. **Clements, J. E., T. Babas, J. L. Mankowski, K. Suryanarayana, M. Piatak, Jr., P. M. Tarwater, J. D. Lifson, and M. C. Zink.** 2002. The central nervous system as a reservoir for simian immunodeficiency virus (SIV): steady-state levels of SIV DNA in brain from acute through asymptomatic infection. J. Infect. Dis. **186:**905–913.
9. **Davis, L. E., B. L. Hjelle, V. E. Miller, D. L. Palmer, A. L. Llewellyn, T. L. Merlin, S. A. Young, R. G. Mills, W. Wachsman, and C. A. Wiley.** 1992. Early viral brain invasion in iatrogenic human immunodeficiency virus infection. Neurology **42:**1736–1739.
10. **Franchini, G.** 2003. Modeling immune intervention strategies for HIV-1 infection of humans in the macaque model. Clin. Appl. Immunol. Rev. **3:**289–306.
11. **Hawke, S., P. G. Stevenson, S. Freeman, and C. R. Bangham.** 1998. Long-term persistence of activated cytotoxic T lymphocytes after viral infection of the central nervous system. J. Exp. Med. **187:**1575–1582.
12. **Hel, Z., J. Nacsa, B. Kelsall, W.-P. Tsai, N. Letvin, R. W. Parks, E. Tryniszewska, L. Picker, M. G. Lewis, Y. Edghill-Smith, M. Moniuszko, R. Pal, L. Stevceva, J. D. Altman, T. M. Allen, D. Watkins, J. V. Torres, J. A. Berzofsky, I. M. Belyakov, W. Strober, and G. Franchini.** 2001. Impairment of Gag-specific $CD8^+$T-cell function in mucosal and systemic compartments of simian immunodeficiency virus mac251- and simian-human immunodeficiency virus KU2-infected macaques. J. Virol. **75:**11483–11495.
13. **Hel, Z., J. Nacsa, E. Tryniszewska, W. P. Tsai, R. W. Parks, D. C. Montefiori, B. K. Felber, J. Tartaglia, G. N. Pavlakis, and G. Franchini.** 2002. Containment of simian immunodeficiency virus infection in vaccinated macaques: correlation with the magnitude of virus-specific pre- and postchallenge $CD4^+$ and $CD8^+$T cell responses. J. Immunol. **169:**4778–4787.
14. **Hel, Z., W. P. Tsai, A. Thornton, J. Nacsa, L. Giuliani, E. Tryniszewska, M. Poudyal, D. Venzon, X. Wang, J. Altman, D. I. Watkins, W. Lu, A. von Gegerfelt, B. K. Felber, J. Tartaglia, G. N. Pavlakis, and G. Franchini.** 2001. Potentiation of simian immunodeficiency virus (SIV)-specific $CD4^+$ and $CD8^+$ T cell responses by a DNA-SIV and NYVAC-SIV prime/boost regimen. J. Immunol. **167:**7180–7191.
15. **Hirsch, V. M., G. Dapolito, P. R. Johnson, W. R. Elkins, W. T. London, R. J. Montali, S. Goldstein, and C. Brown.** 1995. Induction of AIDS by simian immunodeficiency virus from an African green monkey: species-specific variation in pathogenicity correlates with the extent of in vivo replication. J. Virol. **69:**955–967.
16. **Hirschberg, D. L., G. Moalem, J. He, F. Mor, I. R. Cohen, and M. Schwartz.** 1998. Accumulation of passively transferred primed T cells independently of their antigen specificity following central nervous system trauma. J. Neuroimmunol. **89:**88–96.
17. **Horn, T. F., S. Huitron-Resendiz, M. R. Weed, S. J. Henriksen, and H. S. Fox.** 1998. Early physiological abnormalities after simian immunodeficiency virus infection. Proc. Natl. Acad. Sci. USA **95:**15072–15077.
18. **Irani, D. N., and D. E. Griffin.** 1996. Regulation of lymphocyte homing into the brain during viral encephalitis at various stages of infection. J. Immunol. **156:**3850–3857.
19. **Jacobson, S., H. Shida, D. E. McFarlin, A. S. Fauci, and S. Koenig.** 1990. Circulating CD8+ cytotoxic T lymphocytes specific for HTLV-I pX in patients with HTLV-I associated neurological disease. Nature **348:**245–248.
20. **Jassoy, C., R. P. Johnson, B. A. Navia, J. Worth, and B. D. Walker.** 1992. Detection of a vigorous HIV-1-specific cytotoxic T lymphocyte response in cerebrospinal fluid from infected persons with AIDS dementia complex. J. Immunol. **149:**3113–3119.
21. **Jin, X., D. E. Bauer, S. E. Tuttleton, S. Lewin, A. Gettie, J. Blanchard, C. E. Irwin, J. T. Safrit, J. Mittler, L. Weinberger, L. G. Kostrikis, L. Zhang, A. S. Perelson, and D. D. Ho.** 1999. Dramatic rise in plasma viremia after CD8+

T cell depletion in simian immunodeficiency virus-infected macaques. J. Exp. Med. **189:**991–998.
22. **Karin, N., F. Szafer, D. Mitchell, D. P. Gold, and L. Steinman.** 1993. Selective and nonselective stages in homing of T lymphocytes to the central nervous system during experimental allergic encephalomyelitis. J. Immunol. **150:**4116–4124.
23. **Krakowski, M. L., and T. Owens.** 2000. Naive T lymphocytes traffic to inflamed central nervous system, but require antigen recognition for activation. Eur. J. Immunol. **30:**1002–1009.
24. **Kuroda, M. J., J. E. Schmitz, A. Seth, R. S. Veazey, C. E. Nickerson, M. A. Lifton, P. J. Dailey, M. A. Forman, P. Racz, K. Tenner-Racz, and N. L. Letvin.** 2000. Simian immunodeficiency virus-specific cytotoxic T lymphocytes and cell-associated viral RNA levels in distinct lymphoid compartments of SIVmac-infected rhesus monkeys. Blood **96:**1474–1479.
25. **Lackner, A. A., M. O. Smith, R. J. Munn, D. J. Martfeld, M. B. Gardner, P. A. Marx, and S. Dandekar.** 1991. Localization of simian immunodeficiency virus in the central nervous system of rhesus monkeys. Am. J. Pathol. **139:**609–621.
26. **Lackner, A. A., P. Vogel, R. A. Ramos, J. D. Kluge, and M. Marthas.** 1994. Early events in tissues during infection with pathogenic (SIVmac239) and nonpathogenic (SIVmac1A11) molecular clones of simian immunodeficiency virus. Am. J. Pathol. **145:**428–439.
27. **Marcondes, M. C., E. M. Burudi, S. Huitron-Resendiz, M. Sanchez-Alavez, D. Watry, M. Zandonatti, S. J. Henriksen, and H. S. Fox.** 2001. Highly activated CD8(+) T cells in the brain correlate with early central nervous system dysfunction in simian immunodeficiency virus infection. J. Immunol. **167:**5429–5438.
28. **Marten, N. W., S. A. Stohlman, R. D. Atkinson, D. R. Hinton, J. O. Fleming, and C. C. Bergmann.** 2000. Contributions of CD8+ T cells and viral spread to demyelinating disease. J. Immunol. **164:**4080–4088.
29. **Marten, N. W., S. A. Stohlman, and C. C. Bergmann.** 2000. Role of viral persistence in retaining $CD8^+$T cells within the central nervous system. J. Virol. **74:**7903–7910.
30. **Marten, N. W., S. A. Stohlman, W. Smith-Begolka, S. D. Miller, E. Dimacali, Q. Yao, S. Stohl, J. Goverman, and C. C. Bergmann.** 1999. Selection of CD8+ T cells with highly focused specificity during viral persistence in the central nervous system. J. Immunol. **162:**3905–3914.
31. **Marten, N. W., S. A. Stohlman, J. Zhou, and C. C. Bergmann.** 2003. Kinetics of virus-specific $CD8^+$-T-cell expansion and trafficking following central nervous system infection. J. Virol. **77:**2775–2778.
32. **Matano, T., R. Shibata, C. Siemon, M. Connors, H. C. Lane, and M. A. Martin.** 1998. Administration of an anti-CD8 monoclonal antibody interferes with the clearance of chimeric simian/human immunodeficiency virus during primary infections of rhesus macaques. J. Virol. **72:**164–169.
33. **Musette, P., J. F. Bureau, G. Gachelin, P. Kourilsky, and M. Brahic.** 1995. T lymphocyte repertoire in Theiler's virus encephalomyelitis: the nonspecific infiltration of the central nervous system of infected SJL/J mice is associated with a selective local T cell expansion. Eur. J. Immunol. **25:**1589–1593.
34. **Osame, M.** 2002. Pathological mechanisms of human T-cell lymphotropic virus type I-associated myelopathy (HAM/TSP). J. Neurovirol. **8:**359–364.
35. **Osame, M., K. Usuku, S. Izumo, N. Ijichi, H. Amitani, and A. Igata.** 1986. HTLV-I associated myelopathy, a new clinical entity. Lancet **i:**1031–1032.
36. **Pal, R., D. Venzon, N. L. Letvin, S. Santra, D. C. Montefiori, N. R. Miller, E. Tryniszewska, M. G. Lewis, T. C. VanCott, V. Hirsch, R. Woodward, A. Gibson, M. Grace, E. Dobratz, P. D. Markham, Z. Hel, J. Nacsa, M. Klein, J. Tartaglia, and G. Franchini.** 2002. ALVAC-SIV-*gag-pol-env*-based vaccination and macaque major histocompatibility complex class I (A*01) delay simian immunodeficiency virus SIV_{mac}-induced immunodeficiency. J. Virol. **76:**292–302.
37. **Patrick, M. K., J. B. Johnston, and C. Power.** 2002. Lentiviral neuropathogenesis: comparative neuroinvasion, neurotropism, neurovirulence, and host neurosusceptibility. J. Virol. **76:**7923–7931.
38. **Poluektova, L. Y., D. H. Munn, Y. Persidsky, and H. E. Gendelman.** 2002. Generation of cytotoxic T cells against virus-infected human brain macrophages in a murine model of HIV-1 encephalitis. J. Immunol. **168:**3941–3949.
39. **Sasseville, V. G., and A. A. Lackner.** 1997. Neuropathogenesis of simian immunodeficiency virus infection in macaque monkeys. J. Neurovirol. **3:**1–9.
40. **Schmitz, J. E., M. J. Kuroda, S. Santra, V. G. Sasseville, S. A. Simon, M. A. Lifton, P. Racz, K. Tenner-Racz, M. Dalesandro, B. J. Scallon, J. Ghrayeb, M. A. Forman, D. Montefiori, E. P. Rieber, N. L. Letvin, and K. A. Reimann.** 1999. Control of viremia in simian immunodeficiency virus infection by CD8+ lymphocytes. Science **283:**857–860.
41. **Schmitz, J. E., R. S. Veazey, M. J. Kuroda, D. B. Levy, A. Seth, K. G. Mansfield, C. E. Nickerson, M. A. Lifton, X. Alvarez, A. A. Lackner, and N. L. Letvin.** 2001. Simian immunodeficiency virus (SIV)-specific cytotoxic T lymphocytes in gastrointestinal tissues of chronically SIV-infected rhesus monkeys. Blood **98:**3757–3761.
42. **Stevceva, L., B. Kelsall, J. Nacsa, M. Moniuszko, Z. Hel, E. Tryniszewska, and G. Franchini.** 2002. Cervicovaginal lamina propria lymphocytes: phenotypic characterization and their importance in cytotoxic T-lymphocyte responses to simian immunodeficiency virus SIV_{mac251}. J. Virol. **76:**9–18.
43. **Tryniszewska, E., J. Nacsa, M. G. Lewis, P. Silvera, D. Montefiori, D. Venzon, Z. Hel, R. W. Parks, M. Moniuszko, J. Tartaglia, K. A. Smith, and G. Franchini.** 2002. Vaccination of macaques with long-standing SIVmac251 infection lowers the viral set point after cessation of antiretroviral therapy. J. Immunol. **169:**5347–5357.
44. **van der Most, R. G., K. Murali-Krishna, and R. Ahmed.** 2003. Prolonged presence of effector-memory CD8 T cells in the central nervous system after dengue virus encephalitis. Int. Immunol. **15:**119–125.
45. **Veazey, R. S., M. C. Gauduin, K. G. Mansfield, I. C. Tham, J. D. Altman, J. D. Lifson, A. A. Lackner, and R. P. Johnson.** 2001. Emergence and kinetics of simian immunodeficiency virus-specific $CD8^+$T cells in the intestines of macaques during primary infection. J. Virol. **75:**10515–10519.
46. **von Herrath, M., M. B. Oldstone, and H. S. Fox.** 1995. Simian immunodeficiency virus (SIV)-specific CTL in cerebrospinal fluid and brains of SIV-infected rhesus macaques. J. Immunol. **154:**5582–5589.
47. **Williams, K. C., S. Corey, S. V. Westmoreland, D. Pauley, H. Knight, C. deBakker, X. Alvarez, and A. A. Lackner.** 2001. Perivascular macrophages are the primary cell type productively infected by simian immunodeficiency virus in the brains of macaques: implications for the neuropathogenesis of AIDS. J. Exp. Med. **193:**905–915.
48. **Zink, M. C., K. Suryanarayana, J. L. Mankowski, A. Shen, M. Piatak, Jr., J. P. Spelman, D. L. Carter, R. J. Adams, J. D. Lifson, and J. E. Clements.** 1999. High viral load in the cerebrospinal fluid and brain correlates with severity of simian immunodeficiency virus encephalitis. J. Virol. **73:**10480–10488.